

REMARKS

Rejection Under 35 USC 102(b) and (e)

Claims 12 and 13 have been rejected under 35 USC 102 (b) and (e) as being anticipated by Agrawal et al. and Mayrand et al., in turn. In response to this rejection, Applicants' Claims 12 and 13 have been cancelled. The subject matter of cancelled Claims 12 and 13 has been incorporated in new methods Claims 17 and 18.

Rejection Under 35 USC 103(a)

Claims 1, 2, 5-14 and 15 have been rejected under 35 USC 103(a) as being unpatentable over Turnbow et al., in view of Holmstrom et al. and further in view of Parkhurst et al. More specifically, the Patent Office stated that:

It would have been *prima facie* obvious... to combine the RNase protection method of Turnbow with the capture method of Holmstrom and the fluorescent labels of Parkhurst since Holmstrom states "In this report we describe a novel nonradioactive detection system which is rapid as well as sensitive. The handling is easy as it can be carried out in microtiter plates; furthermore, it is easily adapted to other primer sets". An ordinary practitioner would have been motivated to use the capture method of Holmstrom, in which nucleic acid hybridizations were captured with biotin streptavidin linked magnetic beads or microtiter dishes for the advantages expressly noted by Holmstrom including rapid speed, ease of handling and highly sensitive detection. An ordinary practitioner would have been motivated to combine the fluorescent labels of Parkhurst with the RNase protection and capture method of Turnbow in view of Holmstrom since Parkhurst states "the double-labeled oligomer is very effective in signaling hybridization". Parkhurst further notes "Because of this exquisite sensitivity, ^R*oligo*^F may prove to be a very useful tool for investigating the physical behavior of oligomers in solution". An ordinary practitioner would have been motivated to combine the fluorescent labels and FRET technique of Parkhurst with the method of Turnbow in view of Holmstrom for the express advantages of exquisite sensitivity and effectiveness in signaling hybridization as expressly noted by Parkhurst.

This rejection is respectfully traversed. The cited references, taken in combination, do not render obvious Applicants' invention. For example, in one aspect Applicants' invention relates to the use of a unique oligonucleotide probe design to specifically identify predetermined nucleic acid sequences through hybridization of the probe to a pool of nucleic acids sequences, with subsequent cleavage of the probe at sites of base pair mismatch, which eliminates detection of incompletely hybridized probe. As a result, only completely hybridized probe is detected. In one embodiment, a designed linkage includes two marker groups separated by an intervening oligonucleotide. As detailed in the Specification, this design provides improved specificity in identifying target sequence by 1) negating the size fractionation of hybridization products to eliminate background hybridization products and 2) allowing for the use of shorter probes (10 to 100 nucleotides) than had been successfully used in the prior art. Unlike the protection assays of the prior art, detection of partial hybridization is eliminated through digestion of internal base pair mismatches, eliminating the added step of determining hybrid size (usually achieved via gel electrophoresis). Although Parkhurst et al., Turnbow et al., and Holmstrom et al. disclose techniques similar to those utilized in the present invention, none of the above referenced disclosures provide methods which eliminate detection of products from base pair mismatches in hybridized sequences without gel electrophoresis to confirm product size.

Furthermore, Applicants point out that the sensitivity of the Holmstrom et al. detection method, referred to in the above quoted passage, stems from the use of the polymerase chain reaction (PCR), which is not a method used in the present invention. Along the same lines, the "exquisite sensitivity and effectiveness in signaling hybridization" of the Parkhurst et al. method, refers to an application which differs significantly from the present invention. Parkhurst teaches a hybridization signaling method whereby energy transfer from a first label, on

an oligo, to a second label, on the same oligo, is less efficient when the oligo is bound in a DNA duplex hybrid, and this difference in energy transfer is used to detect probe binding in a DNA duplex hybrid. The term "exquisite sensitivity" as used by Parkhurst et al. describes the detection of subtle wavelength changes that result from the oligo passing through different conformations. This is not a method used in the present invention, which teaches the use of fluorescent labels to detect the presence or absence of an intact oligonucleotide.

Furthermore, although highly sensitive to detecting subtle conformational changes of an oligomer, the method of Parkhurst et al. is non-specific for base pair mismatches, which are equally detected (page 288, paragraph 4). These base pair mismatches were not a concern of Parkhurst et al., as their objectives were neither to achieve nor exclusively detect complete hybridization. In light of these particulars, an ordinary practitioner would not have been motivated to create the method of nucleic acid sequence detection of the present invention, by combining the fluorescent labels and FRET technique of Parkhurst et al., with the method of Turnbow et al., in view of Holmstrom et al., because there is no direct relevance of the method of Parkhurst et al. to the methods of Holmstrom et al. and Turnbow et al. to motivate such a combination.

Claims 3 and 4 have been rejected under 35 USC 103 (a) as being unpatentable over the above referenced disclosures and further in view of Thompson et al. and further in view of Mayrand et al. It is respectfully submitted that this rejection is obviated by the above arguments. Neither Thompson et al., nor Mayrand et al., disclosed methods that make obvious the primary advancement of the present invention described above.

Claim 16 has been rejected under 35 USC 103(a) as being unpatentable over Turnbow et al. in view of Holmstrom et al. and further in view of Parkhurst et al. and further in view of Dower et al. Furthermore, with respect to Dower et al. the Patent Office stated that:

Dower states "For instance, one could read the tag directly from the bead by sequencing or hybridization (column 19, line 47-48)". This express teaching motivates the use of hybridization detection methods such as the RNase or S1 nuclease protection methods disclosed.

This rejection is respectfully traversed for reasons discussed above in connection with the rejection of Claims 1, 2, 5-14 and 15 under 35 USC 103(a) in light of the arguments made above regarding Turnbow et al., Holmstrom et al., and Parkhurst et al. The cited Dower et al. reference offers no teachings which cure the deficiencies noted above in connection with these previous rejections.

Summary

In light of the above amendment and remarks, reconsideration of the subject patent application is respectfully requested.

Respectfully submitted,



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